Lasers in Surgery and Medicine 25:43–50 (1999)

Laser Skin Resurfacing Using a Frequency Doubled Nd:YAG Laser After Topical Application of an Exogenous Chromophore

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Background and Objectives: **Although laser skin resurfacing performed with CO_2 or Er:YAG lasers is efficient, side effects such as prolonged postoperative erythema, delayed healing, scarring, and pigmentation, have been reported. These side effects are due to skin characteristics but also to variations of the thermal effects associated with laser skin resurfacing. The study aimed to evaluate a new laser resurfacing method based on a previous topical application of an exogenous chromophore in order to have reproducible thermal effects.**
Materials and Methods: **Exogenous chromophore consisted in carbon dispersed and mixed with film-forming polymers and water. The resultant solution was applied to the skin surface using an airbrush. Experimental evaluation was performed in vivo on hairless rat skin using the following parameters (532 nm, 2.7 W, 1 mm, 50–200 ms, 17.2–68.8 J/cm^2, single pass). Skin biopsies were taken to evaluate histological changes and to quantify epidermis ablation and dermal coagulation depth. Wound healing was followed up during 10 days.**
Results: **Total epidermis ablation was achieved with all pulse durations used. Dermal coagulation depth increased as a function of exposure time. Scar formation was correlated with dermal coagulation depth.**
Conclusion: **The concept of applying a carbon-based solution onto skin in order to obtain laser light conversion into heat followed by heat transfer to the tissue is valid for laser skin resurfacing. By selecting exposure time, the thermal effects are predictable and dermal coagulation depth can be either that observed with a Er:YAG laser or that obtained with a CO_2 laser. Moreover, frequency doubled Nd:YAG laser, already used in dermatology for angiodysplasias treatment, could be easily used for resurfacing of periorbital or perioral zones. Lasers Surg. Med. 25:43–50, 1999.** **© 1999 Wiley-Liss, Inc.**

Key words: skin resurfacing; epidermis ablation; carbon; dermal coagulation; rat; thermal injury

INTRODUCTION

Carbon dioxide (CO_2) laser resurfacing has become recently a very popular method of rhytide and scar removal. Several studies described the

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Accepted 10 March 1999

method, histology, and clinical results of CO_2 laser resurfacing [1–3]. With the development of short-pulsed high-peak power and computerized scanned carbon dioxide (CO_2) lasers, the epidermis can be removed in a precise and reproducible manner leaving only a narrow zone of thermal damage [4,5]. More recently, Er:YAG lasers, displaying a much higher absorption by water, were introduced for this application. They produce less thermal damage with consequently a reduced time of wound healing [6–8]. The role of this thermal damage zone on the outcome of laser resurfacing is not well understood and heat-induced collagen alteration obtained with CO_2 laser is thought to be the basis of the clinical improvement seen in wrinkles treatment [9]. Other infrared lasers, such as Er:YSGG (2,790 nm), Ti:Al_2O_3 (800 nm) or Q-switched Nd:YAG (1,064 nm) have also been used to induce ablation of skin with minimal adjacent thermal damage [10–12].

Many side effects after laser skin resurfacing procedure have been reported: prolonged postoperative erythema, delayed healing, scarring, and dyspigmentation. These side effects are due to several factors: skin characteristics (water content, wound healing ability) and also thermal variation effects induced by laser-tissue interaction (number of passes, laser parameters, handpiece type). The heat transfer to perilesional structures is predominantly a function of pulse width and the interplay between laser wavelength and target tissue absorption characteristics. For the lasers used in skin resurfacing procedure, water is the main chromophore. Water content and its distribution within the tissue is a function of body region, age of the patient, and skin type [13].

Simultaneous control of thermal damage and tissue ablation would be optimal for laser resurfacing. Thus, we assume that the use of a quantified amount of exogenous chromophore could represent an alternative for controlling the laser absorption.

Several studies have demonstrated that the rate and extent of thermal damage can be controlled by adjusting the incident dose of laser energy and the amount of target exogenous chromophore into the tissue [14–16]. Previous work suggests that the control of skin ablation and/or coagulation using ICG (Indocyanin Green) topical application before laser irradiation was better than without ICG application [17]. We decided to apply a similar technique combining a topical suspension of carbon-based solution and the frequency doubled Nd:YAG laser (532 nm), which is widely used in Dermatology for angiodysplasias treatment [18–20].

This study aimed to demonstrate that the severity and extent of thermal damage into the tissue during skin resurfacing procedure could be controlled by adjusting the incident dose of laser energy on exogenous chromophore deposed on skin surface. Thus, we quantified epidermis ablation and dermis coagulation combining a topical suspension of carbon-based solution and a frequency doubled Nd:YAG laser irradiation. Topical solution was assessed by measuring its effect on the absorption and transmission of laser energy on rat skin in vivo. Epidermis ablation depth and dermis coagulation thickness were also evaluated as a function of pulse duration.

MATERIALS AND METHODS

532 nm Nd:YAG Laser

The frequency doubled Nd:YAG laser (Ophtalas FX2, Alcon Biophysic, 532 nm) irradiation was delivered via a 600 μm optical fiber connected to an optical system giving a 1 mm diameter spot. Large area treatments (i.e., greater than 1 mm) were obtained by moving the animal placed on an X, Y table with a micro-manipulator. The power used was 2.7 W giving consequently a 345 W/cm^2 power density. Three different pulse durations, 50, 100, and 200 ms were used. Fluences used were respectively: 17.2 J/cm^2, 34.4 J/cm^2, and 68.8 J/cm^2. Only one single pass was performed without overlapping. The power and the energy output from laser were checked regularly with a power/energy meter (Head: OPHIR L-30-A, Digital electronic: Ophir Laserstar, Ophir Optics Ltd., Jerusalem, Israel) to evaluate variations. These variations represented 2% of power/energy used.

Formulation Investigated

We used carbon particles as exogenous chromophore. Indeed, the carbon has maximum and nearly constant absorption coefficient (μ_{carb}) from UV to near infrared spectrum ($\mu_{carb} \sim 10^5$ cm^{-1} [21]). In order to avoid any penetration of carbon particles into skin, and to apply this exogenous chromophore in a reproducible manner, we developed a specific film. Thirteen nanometer (13 nm) carbon powder particles (Degussa AG, Frankfurt,

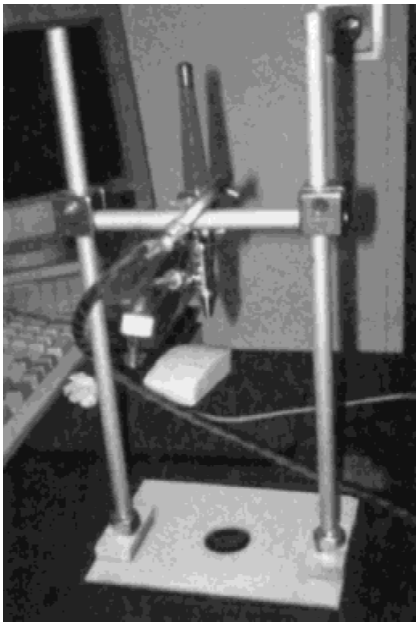

Fig. 1. Formulation-dispensing device.

Germany) were dispersed and mixed with film-forming polymers and water to obtain a final concentration of 0.5% (w/w). This solution was applied to the skin surface using an airbrush. The airbrush was fixed on a support to obtain the same spatial position of the airbrush duct during all carbon/film mixture application procedures (Fig. 1). The quantity of solution applied on the skin was quasi-constant by fixing the solution weight in the airbrush container. After water evaporation, the solution was transformed in a thin film (Fig. 2).

The mean mass of film applied to the tissue was 5.20 ± 0.17 mg (Mean ± SEM; n = 15), determined by weighing glass slides before and after the application and evaporation of solution. The mean density of film was 1.1 g/cm^3, determined by introducing determinate mass of film in known volume container and filling up this container with known density liquid. Since film surface area was 7.3 cm^2, the mean thickness of film was 6.50 ± 0.21 μm (Mean ± SEM; n = 15). The optimal film thickness that prevents laser energy transmission through the film was determined by irradiating formulation sprayed on glass slides with maximal pulse duration (200 ms). The glass slides were placed directly on the thermopile surface (Ophir L-30-A, Ophir Optics Ltd., Jerusalem, Israel) in order to minimize energy losses due to laser beam diffusion through the film.

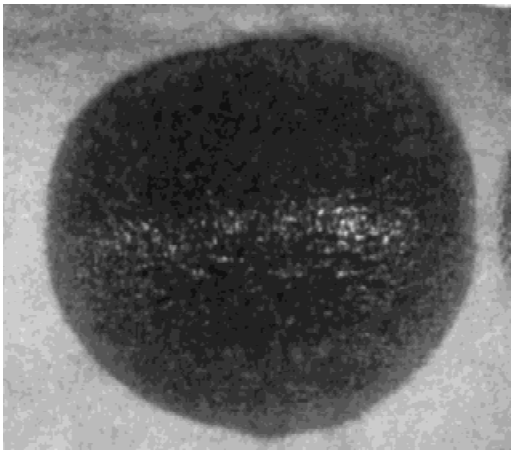

Fig. 2. Carbon particles film dispensed on rat skin.

In Vivo Rat Study

Eight hairless rats were used to evaluate the skin response to exogenous chromophore enhanced 532 nm Nd:YAG laser irradiation. The experiments were performed in accordance with the animal use committee guidelines. Our animal ethics committee approved the protocol. Four hairless rats were used for histological study and four others for clinical wound healing study. Gaseous anesthesia with Forene (Abbott, Rungis, France) were performed to take full effect before applying formulation and irradiation. Three areas were marked on the back of each animal using black ink tattoos (nine points) so that irradiated sites could be identified later. After carbon formulation application and water evaporation, the three areas per animal were irradiated by 532 nm Nd:YAG laser with a 1 mm spot size and pulse duration of 50, 100, and 200 ms respectively (2.7 W, fluence ranging from 17.2 to 68.8 J/cm^2). Each area was treated by positioning animals on micrometric X/Y table (Polytec PI GmbH & Co., Waldbronn, Germany). Forty six juxtaposed pulses per area without overlap were produced for clinical wound healing study, 23 pulses per area for biopsies. After irradiation, the remaining film was removed by using Blenderm (3M France, Cergy Pontoise, France) tape. Biopsies (one per treatment area) were performed on irradiated areas immediately after laser processing. The morphological changes and the extent of thermal damage were characterized by histological study.

For wound healing, the clinical evaluation involved only macroscopic observations. After laser irradiation, the rats were then placed in indi-

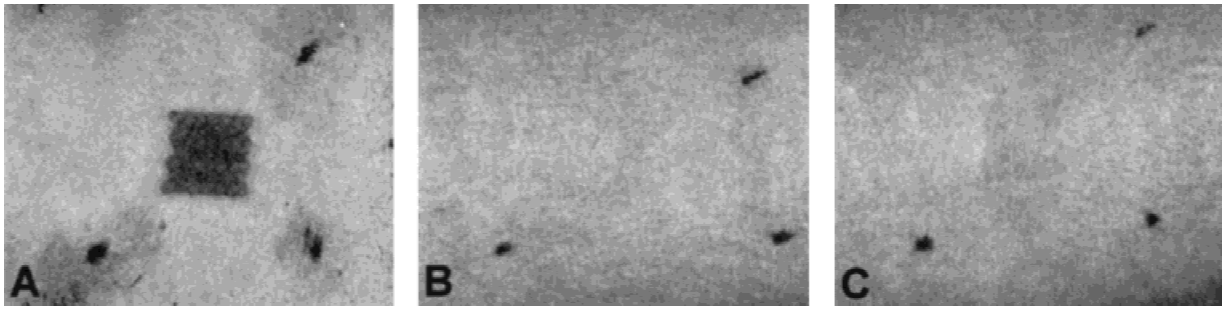

Fig. 3. Macroscopic photographs of wound healing after skin resurfacing using exogenous chromophore (three black ink tattoos were used to identify the treated area). Power = 2.7 W, spot size: 1mm (an area consisted of 46 juxtaposed spots). **A:** Pulse duration: 200 ms, J0; **B:** Pulse duration 50 ms, J6. **C:** Pulse duration 200 ms, J10.

vidual cages without dressing or other wound care on treated areas. Laboratory conditions were controlled: temperature, 21 ± 1°C; relative humidity, 60% ± 15 %; nycthemeral cycle, 12 h/12 h. For each animal, skin reactions were evaluated daily during 10 days. Textural changes were noted and erythema was graded using the Draize scale [22]. Macroscopic pictures of each irradiated area were taken daily.

Histopathological Studies

In order to assess thermal damage, one biopsy per area was taken immediately after laser irradiation, placed into 10% buffered formalin and processed for histological examination (dehydration, paraffin embedding, 5μm-thick sections, and staining). Haematoxylin-eosin was used as topographic staining and Masson's staining (staining kit, Microm, France) to evaluate collagen changes. Histopathological analysis was performed with a BH-2 type Olympus light microscope (Olympus Optical Co., Ltd., Tokyo, Japan) expanded for numeric image acquisition. Morphometric parameters, i.e., depth of ablation craters and extent of thermal damages characterized by dermal and epidermal changes, were quantified using image analysis software (Optimas Corporation, Bothell, WA). For each biopsy, thermal damage depth was blind measured and 18 measures per slide were done. Means and standard error mean (SEM) were calculated for each slide. Linear fit was performed for dermal coagulation depth and pulse duration.

RESULTS

Macroscopic photographs (Fig. 3) illustrated the photothermal ablation of skin combining exogenous chromophore and a frequency doubled Nd:YAG laser.

Immediatly after treatment, all irradiated sites were characterized by a brownish color of superficial tissue (superficial tissue carbonization). For 50 ms pulse duration, residual carbon particles were observed after film removal. The brownish color was darker for a pulse duration of 200 ms (see Fig. 3A) compared with the skin colors observed for 100 ms and 50 ms. Two days later, all irradiated sites showed crusts without erythema. At four days, absence of crusts was noted on all treated sites; however erythema was systematically observed for all animals. Table 1 summarizes the skin reactions observed between day 4 to day 10.

Erythema was dependent on pulse duration: it is very slight for 50 ms (all animals), slight for 100 ms (all animals), and moderate for 200 ms pulse duration (all animals). At six days, no skin reaction was observed on areas treated with a 50 ms pulse duration (see Fig. 3B). At eight days, a similar evolution was observed on sites treated with a 100 ms pulse duration.

At 10 days, no erythema was noted. On the sites treated with a 200 ms pulse duration, the following observations corresponding to a scar formation were done: slight depression, whiteness, shininess, and loss of normal skin surface marking (see Fig. 3C). Chronological wound healing evaluation has showed that moderate erythema noted at day 4 led to scar formation 10 days after irradiation.

Biopsies histological examination showed that the 532 nm laser with a power of 2.7 W combined with carbon particles film and pulse duration ranging from 50 ms to 200 ms induced tissue ablation (mostly epidermis and upper part of the dermis) with thermal injury, below and within the

TABLE 1. Chronological Skin Reaction Evaluations on Rat Skin After 532nm Laser Irradiation Using a Carbon Particles Film.

	Pulse duration		
	50 ms	100 ms	200 ms
4 Days	Very sight erythema (4/4)	Slight erythema (4/4)	Moderate erythema (4/4)
6 Days	— (4/4)	Very slight erythema (4/4)	Slight erythema (4/4)
8 Days	— (4/4)	— (4/4)	Slight erythema (4/4)
10 Days	— (4/4)	— (4/4)	No erythema Scar formation (4/4)

Power = 2.7 W.—: Normal skin (no erythema and no scar formation). The frequency of observations is indicated between brackets.

surrounding epidermis. The dermal changes consisted in a band of collagen denaturation. With haematoxylin-eosin staining, this band of denatured collagen was characterized by the compact pattern of collagen fibers with glassy appearance and more intense pink staining (Fig. 4A–C). With Masson's staining, the band of denatured collagen was characterized by change of staining affinity. With our staining procedure, collagen appeared reddish in irradiated areas whereas it appeared green in other sites (Fig. 4C–E). Moreover we observed moderate vacuolation of keratinocytes within the epidermis immediately adjacent to the irradiated zones.

Tissue morphometry was done to quantify observed histological changes. In this animal model, normal epidermis thickness is between 30 μm and 50 μm. The depth of ablated tissue and depth of surrounding thermal changes were shown to be dependent of pulse duration. For a 50 ms pulse duration, ablation concerned only epidermis (less than 25 μm). For 100 ms pulse durations, ablation depth ranged from 25 to 50 μm. For 200 ms pulse duration, ablation reached 50 μm to 75 μm. Residual thermal necrosis was thinner for a 50 ms pulse duration (approximately 60 μm) compared to longer pulse durations: 105 μm in mean (100 ms) and 185 μm (mean for 200 ms) (Fig. 5). Linear fit showed a very good correlation (R = 0.9973) between dermal coagulation depth and pulse duration.

DISCUSSION

The aim of these experiments was to evaluate a new technique for laser skin resurfacing. The originality of this technique was to combine a 532 nm Nd:YAG laser with a carbon particle film previously applied topically on skin. This laser technique is based on the absorption of laser energy by the exogenous chromophore contained in the thin film. Due to the high absorption of carbon particles at 532 nm wavelength ($\mu_{carb} \sim 10^5$ cm^{-1} [21]), the light is very efficiently converted into thermal energy. This new concept is based on standardization of skin thermal damage during laser skin resurfacing by controlling the heat source. Indeed, if the film thickness and its absorption coefficient at the laser wavelength were standardized, the thermal process (heat transfer by thermal conductivity and explosive tissue vaporization) depended only on laser parameters. For this study, the resurfacing was achieved with a single pass (P = 2.7 W, spot size: 1 mm, pulse duration ranging from 50 ms to 200 ms, fluence ranging from 17.2 J/cm^2 to 68.8 J/cm^2). Since the film was standardized: (1) composition, (2) film thickness, (3) carbon concentration as well as the laser power density were all fixed, this study was limited to the evaluation of three different pulse durations on the severity and extent of thermal damage leading to epidermis ablation and dermis coagulation.

In dermatology, the 532 nm Nd:YAG laser is preferentially used to treat vascular lesions since the 532 nm wavelength is well absorbed by hemoglobin. In order to avoid any vascular injury, the film was designed to convert 100% of laser energy into thermal energy. The minimal film layer thickness was determined to avoid laser energy transmission through the film for 2.7 W. The histopathological studies confirmed the absence of transmission of laser light into the dermis since no vascular damage was observed. Moreover, these histopathological studies showed that there was no penetration of carbon particles into the stratum corneum.

Although the rat skin is different from the human skin (the epidermis is thinner than human epidermis and papillary dermis is absent), it appears to be well adapted for the initial experi-

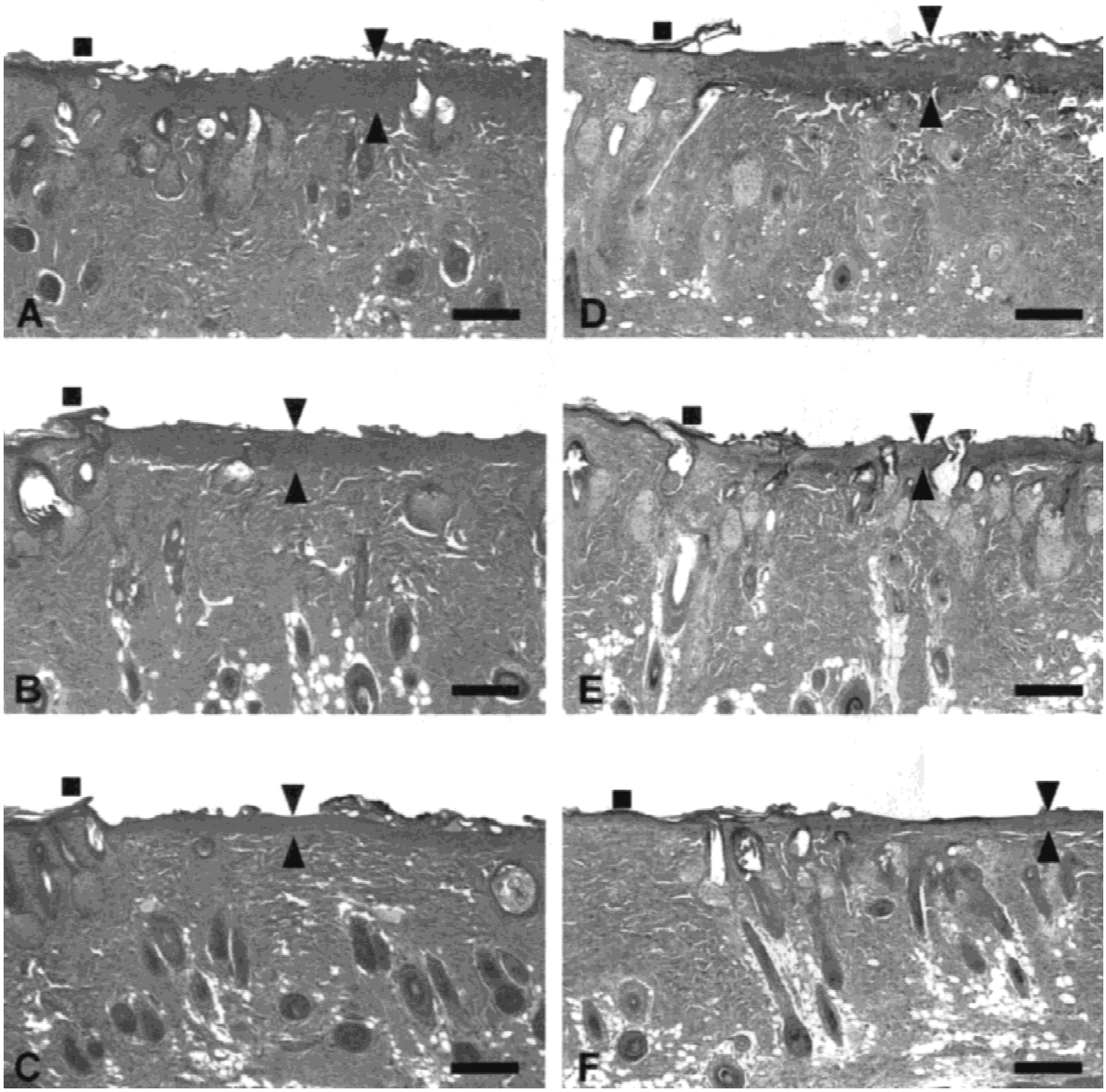

Fig. 4. Comparative light-microscopic views of adjacent thermal tissue damage produced by 532 nm Nd:YAG laser combined with a carbon particles film on rat skin in vivo. Coagulation zones are outlined by arrowheads. Squares denote the interaction limits. Size bar = 200 μm. Power = 2.7 W, spot size: 1mm. **A–C:** H-E staining; **D–F:** Masson's staining. A,D: Pulse duration = 50 ms; B,E: Pulse duration = 100 ms; C,F: Pulse duration = 200 ms.

mental evaluation of the laser resurfacing technique. Since the laser power and the film thickness were fixed, our results indicated that the ablation severity and depth of surrounding thermal changes varied only with pulse duration. In our experimental conditions (film thickness = 6.50 ± 0.21 μm, P = 2.7 W, spot diameter = 1 mm, Gaussian beam, rat skin), dermal coagulation depth was linearly related to function of pulse duration (R = 0.9973) (Fig. 5).

These results are comparable to those obtained on pig skin using CO_2 or Er:YAG laser. For example Fitzpatrick [23] using a pulsed CO_2 laser, observed dermis coagulation ranged from 53 μm up to 106 μm (two and three passes) and increasing with pulse energy and numbers of passes. In a similar study using Er:YAG, Kaufmann [6] showed dermis coagulation ranging from 40 μm (300 mJ) to 50 μm (600 mJ). In our study, on rat skin, dermis coagulation was noted from 60 μm (50 ms) up to 185 μm (200 ms) and increased linearly with pulse duration.

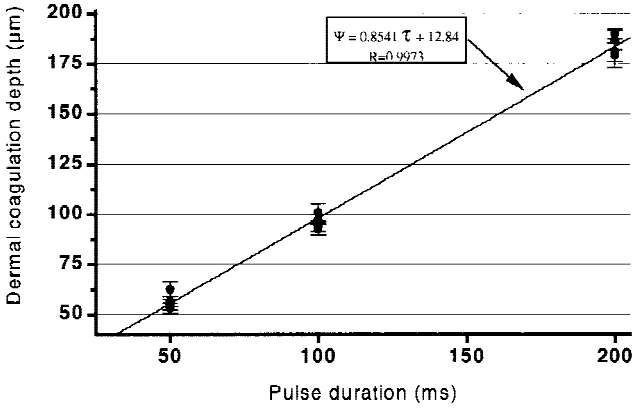

Fig. 5. Average depth of dermal coagulation in four hairless rat skin blind-measured on skin histological preparations after 532 nm Nd:YAG laser irradiation combined with a carbon particles film. Power = 2.7 W, spot size: 1mm. Mean dermal coagulation depth ± SEM (n = 18, 18 measurements per slice) is plotted as a function of pulse duration. Straight line fit is from a linear regression.

The human facial epidermis is thicker than rat epidermis (approximately 1.5 times). According to our results, we assume that the depth of thermal injury will be lower in human skin than in rat skin using the same parameters. However, further studies are needed to confirm this assessment and to evaluate the wound healing process in human with our skin laser resurfacing concept.

CONCLUSION

In conclusion, the concept of applying a carbon-based solution onto skin in order to obtain laser light conversion into heat followed by heat transfer to the tissue is valid for laser skin resurfacing. By selecting exposure time, the thermal effects are predictable and dermal coagulation depth can be either that observed with a Er:YAG laser or that obtained with a CO_2 laser. If application of the film with an airbrush is efficient, this technique is not adapted to a clinical approach and could be improved by manufacturing a film where all parameters: thickness, chromophore concentration, and chromophore distribution, will be standardized. Moreover, frequency doubled Nd:YAG lasers already used in dermatology for angiodysplasias treatment could be easily used for resurfacing of periorbital or perioral zones.

ACKNOWLEDGMENT

The authors thank Mr. L. Moussiere from ALCON, France for the loan of 532 nm Nd:YAG laser.

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